channel is impermeable to ions and water (Saparov et al., 2007). Here we have tested whether the channel remains a barrier to small molecules after a conformational transition occurred due to ligand binding. Therefore, we reconstituted the purified and fluorescently labeled translocation channel SecYEG into planar lipid bilayers. Positioning of the membrane on top of a laser scanning microscope with single molecule sensitivity allowed monitoring the reconstitution efficiency. Both the motor molecule SecA and ribosomes induced ion channel activity. The probability of the channel opening was derived from the number of open channels and the total number of reconstituted channels.

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Reference

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728-Pos Board B528

Effect of Relative Humidity on the Permeability of Model Skin Lipid Membranes

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Skin stratum corneum (SC) lipids play a very import role in formation of skin barrier, for instance, in controlling water transport. However, very little research effort was focused on understanding the mechanism of water permeability across the lipid bilayer stacks. In this study we quantitatively investigated the permeability and the responding behavior of model skin lipid membranes at different values of relative humidity (RH) using quartz crystal microbalance (QCM). We show that the diffusion constant of water across the membrane has a maximum at RH=40-50%, whereas the equilibrium content of water within the membrane increases monotonically with increasing humidity. The permeability of membrane also increases with RH, showing that the membrane is responsive rather than passive, as proposed by E. Sparr et al. (Soft Matter, 2009, 5, 3225). A small amount of Oleic acid used as a penetration enhancer in drug delivery, causes the permeability of model membranes to increase; this increase is especially pronounced at high humidity. The effect of water sorption on high-frequency viscoelastic properties of skin lipid membranes is also discussed. It is shown that the elastic modulus of the membrane decreases and the loss modulus increases as a result of water sorption.

729-Pos Board B529

Effects of Temperature on Membrane Electrodiffusion

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From a theoretical point of view many cell functions depend on molecular transport across their membrane. Cell nutrition, action potential generation, and general signaling are examples of this. In this work we aim to increase our understanding of how the properties of membrane transport depend on temperature. To address this problem, we take a biophysical approach using the Nernst-Planck equation to model molecular flow across the membrane. With this equation we model two processes of high relevance: cellular excitability and regulation of extracellular pH. We use dynamical systems theory to analyze the behavior of the system with respect to temperature. We specifically address the following questions: does temperature affect the dynamics of action potential generation and firing frequency? What is the dependence of the transmembrane acidity levels on body temperature? In regard to excitability, we expect to describe the behavior of neurons and other excitable cells in cold blooded animals as the environmental temperature changes. In addition, one potentially interesting aspect of our findings in regard to pH regulation is to unravel mechanisms explaining why temperature-based therapies work for the treatment of some cancers.

730-Pos Board B530

Sodium-Coupled Substrate Translocation Mechanism via the Sodium/Betaine Symporter

Paween Mahinthichaichan.

The sodium/betaine symporter or BetP, a member of the betaine/choline/carnitine transporter family and a LeuT-fold symporter, co-transports two Na+ ions along with its substrate betaine in response to the intracellular hyperosmotic stress. Its substrate-bound crystal structure is occluded to the extracellular lumen but is likely open to the cytoplasmic lumen via an observable pathway. Even though no ions were reported in the crystal structure, the binding sites of two Na+ ions, so-called Na1 and Na2, have been proposed. In the model, Na1 interacts with the substrate whereas Na2 is bound outside the binding pocket. Unlike Na1, Na2 binds weakly to its nearby residues, as suggested by intermolecular distance of more than 3 Å, so it is unclear whether Na1 and Na2 bind to their putative sites. To test the Na+-binding model and to possibly investigate their roles in substrate translocation, we performed molecular dynamics (MD) simulations on the substrate-bound BetP immersed in membrane in the presence and absence of Na1 and Na2. All short-initial simulation trials demonstrated the rapid formation of a hydrated pathway toward the Na+-binding sites followed by rapid dissociation of Na2 via the same pathway, indicating the opening of the binding pocket to the cytoplasmic lumen and the unstable Na2 binding. Surprisingly, after 38 ns, we observed the coupled displacement of Na1 and substrate, both of which dissociated via the hydrated pathway at the end of the 57-ns simulation. No substantial displacement of the substrate occurred without its first being bound to a Na+ ion, which implicates the Na+-dependent substrate translocation. No major protein conformational changes were also observed despite the dissociation of the substrate. Our findings, especially the coupled Na1-substrate dissociation, provide mechanistic insights into the ion-coupled substrate translocation in BetP and other ion-coupled symporters.

731-Pos Board B531

Effects by Ibogaine on the Dopamine Transporter

Walter Sandtner, Simon Bulling, Klaus Schicker, Chrisitan Gruber, Harald Sitte.

Ibogaine, an alkaloid extracted from Tabemanthe iboga, is being studied as a potential long- acting treatment for opioid and stimulant abuse as well as for alcoholism and smoking. The dopamine transporter (DAT) is a key pharmacological target that modulates the activity of drug reward circuits. Ibogaine is reported to be a competitive inhibitor of DAT and in agreement with this view it enhances similar effects of stimulants (1) (cocaine and amphetamines).

Here we studied how ibogaine affects the electrophysiological properties of DAT. In dopamine transporters, transport is coupled to the sodium gradient and in each transport cycle two positive charges enter the cell. Therefore transport of substrate in DAT is intrinsically electrogenic.

However DAT also carries so called ‘‘uncoupled currents’’ (2), supported by a channel mode that can be adopted by the transporter. In the absence of substrate, uncoupled currents are reflected as a transporter specific leak current (a sodium leak or a lithium leak) and in the presence of substrate uncoupled currents are responsible for about 50% of the total charge flux. We expressed DAT in Xenopus laevis oocytes and characterized the effects of ibogaine on the substrate induced current, the sodium leak as well as the lithium leak.

Our observations are in support of a model in which the channel mode responsible for the uncoupled currents is adopted when the transporter is in an inward facing conformation.

Thus we provide evidence that the different uncoupled currents described in DAT have a mutual basis and that the current can be perceived as a conformational probe.

732-Pos Board B532

Lithium Stabilizes the Inward Facing Conformation of Human Serotonin Transporter (hSERT)

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According to the alternate access model of transport, a transporter such as hSERT adopts two major conformations: the outward facing conformation and the inward facing conformation. For a functional transporter the distribution of these states in equilibrium (with and without substrate) is unknown. Furthermore it is unclear how this distribution is affected by various parameters such as the temperature, the external and internal ion composition, the membrane potential etc.

Here we tested the effect of various external cations such as sodium, lithium and NMDG on the conformational equilibrium.

In this study we probed the conformational equilibrium using three different techniques. First we utilized intramolecular FRET measurements. This was done utilizing a construct of hSERT that had been genetically modified to contain a CFT at the N-terminal end and a YFP at the C-terminal end (CSERTY). CSERTY was heterologously expressed in HEK cells and FRET changes upon administration of different external ions were correlated with the data we obtained from binding of a radio-labeled inhibitor of SERT (second technique) as well as with the data we obtained by testing the accessibility of cysteines introduced by site directed mutagenesis to MTS reagents (third technique). Binding was conducted in membranes of HEK cells that stably expressed hSERT and for the accessibility studies we employed hSERT expressed in Xenopus laevis oocytes.

In accordance with previous studies we find that external sodium supports the outward facing conformation. However for high external lithium concentrations we have evidence that the prevalent conformation is inward facing.